Amendments to the Drawings:

The attached replacement sheets of drawings replace the original sheets of Figs. 1-4. No new matter has been added by these replacements, the applicants are providing formal drawings of the figures originally filed with this application.

Attachments following last page of this Amendment:

Replacement Sheet (4 pages)

REMARKS

Claims 1-24 are pending in this application. Claim 15 has been withdrawn. Applicants have amended claim 6. Support for this amendment exists in the specification, for example, at page 10, lines 1-14.

Drawings

Applicants submit herewith replacement sheets with formal drawings of Figures 1 to 4. Applicants submit that these replacement sheets overcome the objections to Figures 3 and 4. No new matter has been added by the replacement sheets.

Response to Restriction

Applicants respectfully maintain their traversal of the restriction requirement and request that the Examiner reconsider and withdraw the restriction requirement with respect to Groups I and II. Claim 15, the sole claim of Group II, depends from claim 12 (Group I) and should be included in the same group as claims 1 and 12.

Claim 12 reads "A composition comprising an isolated, single-chain pro-urokinase ("pro-UK") mutant polypeptide produced according to the method of claim 1, wherein at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide."

Claim 15 depends from claim 12 and reads, "The polypeptide of claim 12, wherein the pro-UK mutant is M5."

Claim 12 recites a composition that contains a polypeptide. Claim 15 merely specifies a particular pro-UK mutant polypeptide that can be in the polypeptide-containing composition of claim 12. Accordingly, applicants submit that claim 15 should be included in the same group as claims 1 and 12 (Group I).

The Office Action states at page 2, "Group I is directed to a method of preparing the pro-UK and the MPEP states that if the product can be shown to be of use in a materially different process, the restriction requirement is proper." Applicants submit that if it is proper for claim 12, which recites a product made by the process of claim 1, to be grouped with claim 1, it is also

proper for claim 15 to be grouped with claims 1 and 12, because claim 15 merely specifies a particular mutant polypeptide of claim 12. Therefore, applicants again respectfully request that claim 15 be rejoined with Group I.

Duplicate Claims

The Office Action alleges that claims 21-24 are substantial duplicates of claims 1-3 and 8. Applicants respectfully submit that the claims are not duplicates because a difference lies in who the infringer is in each claim, and the steps recited in claims 1 and 21 indicate who an infringer could be. For example, to infringe claim 1, a person would have to obtain a nucleic acid molecule that encodes a pro-urokinase mutant polypeptide, insert that nucleic acid into a pET29a expression plasmid, use that plasmid to transform the BL21/DE3 RIL strain of bacteria, culture the transformed bacteria, and isolate the polypeptide. In contrast, to infringe claim 21, a person would have only to obtain bacteria that have already been transformed with a pET29a expression plasmid that contains a pro-urokinase mutant polypeptide, culture the transformed bacteria, and isolate the polypeptide. The steps differ between these claims, indicating that these claims are not substantial duplicates of each other.

35 U.S.C. § 112, 1st Paragraph: Written Description

The Office Action alleges that claims 12-14 and 16-18 are not described in the specification in a way to reasonably convey to one skilled in the art that the inventors had possession of the composition described in these claims. Applicants respectfully submit that the specification adequately describes the compositions recited in the claims, and request that this rejection be withdrawn.

As an initial matter, applicants respectfully point out that according to MPEP § 2163(II)(A):

> The examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed.

Applicants submit that the specification provides sufficient disclosure to one of ordinary skill in the art to understand the applicants had possession of the invention. For example, claim 12 recites a composition that contains an isolated, single-chain pro-urokinase ("pro-UK") mutant polypeptide produced according to the method recited in claim 1, wherein at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide. Claim 13 recites that at least 98% of the protein in the composition is the pro-UK mutant polypeptide. Claim 14 recites that the pro-UK mutant is a pro-UK flexible loop mutant. Claim 16 recites that the composition can contain a pharmaceutically acceptable excipient; and claim 17 recites that this excipient is acidic. Claim 18 recites a composition containing a certain amount of a pro-UK mutant made by the method recited in claim 1, which is packaged with instructions for administering the composition to a patient exhibiting symptoms of stroke or heart attack.

On page 4, the specification states:

> In other aspects, the invention includes purified pro-UK mutant polypeptides, such as flexible loop mutants, e.g., M5 (both as described herein), produced according to the methods described herein. The isolated pro-UK mutant polypeptides have a purity of 96% or greater, i.e., they are in compositions in which at least 96, 97, 98, or even 99% of the protein in the composition is the single-chain pro-UK mutant polypeptide. The invention also features compositions including pro-UK mutants made according to the new methods and an excipient, e.g., an acidic excipient, as well as a composition including an aliquot of 20-40 mg of a pro-UK mutant made according to any the new methods, packaged with directions for use in administering as a bolus to a patient exhibiting symptoms of a stroke or a heart attack.

The level of disclosure is sufficient for a skilled practitioner to realize that the applicants were in possession of the compositions recited in the claims. Pages 11-14 provide detailed explicit protocols for how to purify the polypeptide recited in the claims. The protocols on these pages provide details on exactly how to obtain a bacterial pellet, how to refold the polypeptide, how to concentrate the polypeptide by ultracentrifugation, how to perform cation exchange chromatography, anion exchange chromatography, hydroxyapatite chromatography, gel filtration chromatography, buffer exchange, and freeze drying. For each of these steps, the protocols

provide buffer recipes including pH, centrifugation conditions including spin times and temperatures, details on sample preparation for each step, incubation conditions, chromatography conditions including buffers and volumes to use, and ways to measure sample purity and concentration. Page 14 describes that the recited compositions can be stored in a pharmaceutically acceptable excipient and provides examples of suitable excipients (organic acids, such as acetic acid) and an example of an appropriate pH of such an excipient (about pH 5.4).

Thus, in reading the specification, a skilled practitioner would understand that the applicants were in possession of the compositions recited in the claims; this is especially true in light of the fact that the level of skill in the field of protein purification is high.

With respect to claim 18,[1] the Office Action states (at page 5):

> The claims are also directed to a composition comprising the mutant made in the method and to packaging with directions said composition for use in administering a bolus to a patient exhibiting symptoms of a stroke or a heart attack (see for example claim 19 [18]). The specification provides no disclosure of a kit or demonstrate a method of treating stroke or a heart attack with the mutant made. Absent guidance/direction on how to use the claimed composition in a medicament and showing said composition being effective to treat the claimed diseases, the instant specification lacks adequate written description ...

Applicants respectfully submit that it is well known in the art to package a composition with instructions for how to use the composition, and as a result, additional guidance as to this element of claim 18 is not required (see MPEP § 2163(II)(A)(2): "Information which is well known in the art need not be described in detail in the specification.").

Claim 18 recites a composition containing an aliquot of a pro-UK mutant that can be administered as a bolus to a patient exhibiting symptoms of stroke or heart attack. Pages 14-15 of the specification provide a detailed description of how to prepare and use a bolus of a pro-UK mutant, and the amount of a pro-UK mutant to include in a bolus. Page 15 even provides examples of situations and describes persons who could use such a bolus and in what situations.

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[1] As a point of clarity, applicants note that the Office Action refers to claim 19 when describing the claim that recites administering a bolus of a pro-UK composition to a patient exhibiting symptoms of stroke or heart attack. Applicants assume that the Office Action is referring to claim 18.

Thus, applicants respectfully submit that the composition recited in claim 18 is adequately described in the specification to satisfy the written description requirement.

Applicants respectfully request that the rejection of claims 12-14 and 16-18 for an alleged lack of written description be withdrawn.

35 U.S.C. § 112, 2nd Paragraph

The Office Action alleges (at page 5) that claim 6 is indefinite for use of the term "containing a sufficient amount of kanamycin."

Without conceding the correctness of this rejection and merely to accelerate prosecution, applicants have amended claim 6 to recite, "wherein the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin to provide selection for kanamycin resistance " to specify that antibiotic resistance (e.g., kanamycin resistance) is selected for during the cell culturing. Applicants submit that this amendment overcomes the § 112, 2nd paragraph rejection and request that this rejection be withdrawn.

35 U.S.C. § 103

The Office Action alleges that claims 1, 5, and 21 are obvious in light of Orsini et al. (*Eur. J. Biochem.*, **195**:691-697 (1991)) and Yuming et al. (*Chinese J. Biotechnol.*, **13**:233-238 (1998)). Applicants respectfully submit that the Office Action has failed to establish a *prima facie* case of obviousness, because the prior art references fail to describe or suggest all the claim limitations, fail to provide a reasonable expectation of success, and fail to suggest their combination.

The methods recited in claims 1, 5, and 21 require the use of the BL21/DE3 RIL strain of bacteria. The Office Action at page 7 states that, "Orsini et al teach a mutated pro-UK expressed in *E. coli* B strain" and "Yuming et al. teach a pro-UK expressed in *E. coli* BL21(DE3)pLyss." Orsini only broadly describes the use of "an *E. coli* type-B strain" (page 692). Yuming describes the use of only the "*E. coli* BL21(DE3)Plyss" strain (page 234). Neither reference describes the

use of a specific strain of type-B *E. coli*: the BL21/DE3 RIL strain recited in applicants' claims. Further, neither reference suggests the use of this specific strain.

As described in the instant application, part of the invention disclosed therein stems from the discovery that higher yields of a pro-urokinase ("pro-UK") mutant polypeptide can be obtained if the BL21/DE3 RIL strain is used to prepare the pro-UK mutant. This was unexpected and, prior to this discovery by the applicants, there was no appreciation that a strain specificity for the expression of a pro-UK mutant existed.

As shown in Example 2 at page 17, the same pro-UK encoding expression plasmid (pET29a) was used to transform three different type-B strains of bacteria, and the yield of pro-UK mutant polypeptide produced by each strain was measured. The BL21/DE3 RIL strain yielded 4.12 grams/liter; in contrast, the BL21(DE3) strain and the BL21(DE3)pLys strain (the same strain described by Yuming) yielded only 0.91 and 0.82 grams/liter, respectively. These results show that the BL21/DE3 RIL strain yielded about 4.5 times as much protein as the other strains. Neither Orsini nor Yuming, alone or in combination, offers any suggestion that such a strain specificity could have existed. Thus, because neither of the cited references describes or even suggests the use of the BL21/DE3 RIL strain to produce the pro-UK mutant polypeptide, the references fail to support the obviousness rejection.

The Office Action alleges (at page 7):

> Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to have a method of preparing a pro-UK mutant polypeptide by inserting the nucleic acid encoding said protein into an expression plasmid comprising a T7 promoter and Shine-Dalgarno sequence and transforming *E.coli* type B strain bacteria, because Orsini et al. teach a mutated pro-UK expressed in *E. coli* B strain having a Shine Dalgarno sequence utilizing a tryptophan promoter. Although the reference is silent on "a T7 promoter", one of ordinary skill in the art knows that the promoter controls growth and allows for high level expression and Yuming et al. teach a pro-UK expressed in *E. coli* BL21(DE3)pLysS under the control of the T7 promoter. One of ordinary skill in the art would be motivated to combine the teachings of the reference because Yuming et al. teach that the expression level of the recombinant pro-UK attained up to 15% of the total bacterial proteins. Further, it is obvious to modify the method of Orsini et al. by substituting the T7 promoter of Yuming with a reasonable expectation of success, because Yuming et al. teach that the previous

> expression level of pro-UK was 2% and that under the control of the T7 promoter a high level of expression is achieved (see page 238).

First, applicants point out that the methods recited in the claims provide a high yield of the pro-UK mutant, because it is the combination of the elements recited in the claims that allows the high yield to be produced. This combination includes the use of the specific BL21(DE3) RIL strain of bacteria. Neither of the cited references discloses or suggests this specific strain. Also, the methods recite the use of a specific expression plasmid to transform the BL21(DE3) RIL bacteria: the pET29a plasmid. Neither of the cited references discloses or suggests this specific plasmid.

Even if the cited references did disclose or suggest the specific elements recited in the claims (which they fail to do), the combined references must suggest the combination of the prior art teachings and that such a combination would have a reasonable likelihood of success. That it may be "obvious to try" such a combination is not the proper standard in an obviousness analysis. Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1380 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987) (an invitation to try an experiment is not the proper test for obviousness); In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529 (Fed. Cir. 1988):

> The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art ...(Citations omitted) Both the suggestion and the expectation of success must be found in the prior art, not in the applicant's disclosure.

The Office Action alleges that a reasonable expectation of success and a motivation to combine the references exist because Yuming states that use of the T7 promoter to drive pro-UK expression increased the yield of pro-UK to 15% of the total bacterial proteins. Applicants submit that this does not lead to a reasonable expectation of success in combining the cited references to arrive at the claimed methods. Applicants' methods are not based solely upon the use of the T7 promoter to drive expression. The claimed methods do not recite only the use of the T7 promoter to achieve increased protein yields. Rather, the methods are based on the combination of the elements recited in the claims. As shown in Example 2 on page 17, the yield

of protein was about 4.5 fold greater when the BL21(DE3) RIL strain of bacteria was used instead of another strain. As discussed above, in each of the three strains tested, the same plasmid, which contained the T7 promoter, was used, and yet the protein yields differed about 4.5 fold. Thus, just because the protein yield in Yuming increased when the T7 promoter was used instead of a different promoter, this does not provide one of skill in the art with a reasonable expectation that using a different bacterial strain would increase yields or with a motivation to combine the references.

Applicants submit that neither of the references cited in the Office Action, alone or in combination, suggest the methods recited in claims 1 (and its dependent claim 5) and 21. Thus, applicants respectfully submit that these references do not render the methods recited in the claims obvious, and request that this rejection be withdrawn.

The Office Action next alleges that claims 1-5, 19, and 21-23 are obvious in light of Orsini, Yuming, and Liu et al. (*Circulation Res.*, **90:**757-763 (2002)). Applicants respectfully submit that the Office Action has failed to establish a *prima facie* case of obviousness, because the prior art references fail to describe or suggest all the claim limitations, fail to provide a reasonable expectation of success, and fail to suggest a combination of the prior art references.

The methods of claims 1 and 21 have been described above. Claim 19 recites a purified culture of BL21/DE3 RIL that contains an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide.

The descriptions of Orsini and Yuming are provided above. Liu describes the use of "an *E. coli* type-B strain" (page 758). None of the three cited references describes the use of the BL21/DE3 RIL strain. Further, none of the references suggests the use of this specific type-B strain. As described above, applicants discovered that the use of the specific type-B strain, the BL21/DE3 RIL strain, led to dramatically increased yields of pro-UK mutant polypeptide. None of the cited references offers any suggestion that such a strain specificity could have existed and thus these references fail to disclose or suggest all of the elements recited in claims 1-5 and 21-23. The references also fail to describe or suggest the purified culture of BL21/DE3 RIL that

contains an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide recited in claim 19.

The Office Action characterizes Orsini and Yuming as above, and alleges at page 9 that "Ning et al. [Liu] teach pro-UK expressed in *E. coli* B strain subjected to site directed mutagenesis producing a mutation of Lys300-His (claims 3 and 23)." The Office Action alleges at page 9 that it would have been obvious to one of ordinary skill in the art to "have a method of preparing a pro-UK mutant polypeptide" for the same reasons presented above and at page 7 of the Office Action.

Applicants respectfully disagree. With respect to the Office Action's description of Orsini and Yuming, applicants have characterized these references above. Liu does not make up for the deficiencies of Orsini and Yuming. This reference fails to describe or suggest use of the BL21(DE3) RIL strain of bacteria, fails to suggest the combination of the references and fails provide a reasonable expectation of success if such a combination was made.

Even if the cited references describe or suggest the specific elements recited in the claims (which they fail to do), to support the rejection they must also suggest the combination of the prior art teachings and that such a combination would have a reasonable likelihood of success. The Office Action alleges at page 9 that a reasonable expectation of success and a motivation to combine the references exist because Yuming states that use of the T7 promoter to drive pro-UK expression increased the yield of pro-UK to 15% of the total bacterial proteins. Again, applicants submit that the claimed method does not rely solely on the use of the T7 promoter; rather it is the combination of elements that leads to applicants' unexpected results of increased protein yields. As discussed above, the fact that protein yield in Yuming increased when the T7 promoter was used instead of a different promoter does not provide one of skill in the art with the motivation to use a different, specific bacterial strain (BL21(DE3) RIL) or with a reasonable expectation of success that using this different, specific strain would increase protein yields even more. That it may be "obvious to try" such a combination is not the proper standard in an obviousness analysis, as discussed above.

Applicants respectfully submit that these references, alone or in combination, do not render the methods recited in the claims 1-5 and 21-23 or the culture recited in claim 19 obvious, and request that this rejection be withdrawn.

Claim Objections

The Office Action has objected to claims 7-11, 20, and 24 because they depend from rejected base claims. Applicants submit that the arguments and amendments presented herein have overcome the rejections of the base claims, thereby overcoming the objections to claims 7-11, 20, and 24.

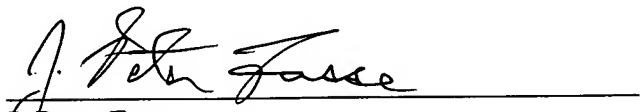
CONCLUSION

Applicants submit that the rejections and objections raised by the Office Action have been overcome by the amendments and arguments made herein, and request allowance of the pending claims.

No fees are believed due. Please apply any charges or credits to deposit account 06-1050, referencing Attorney Docket No. 15702-003001.

Respectfully submitted,

Date: September 6, 2005



J. Peter Fasse
Reg. No. 32,983

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

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